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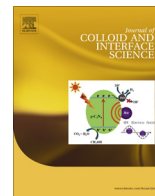
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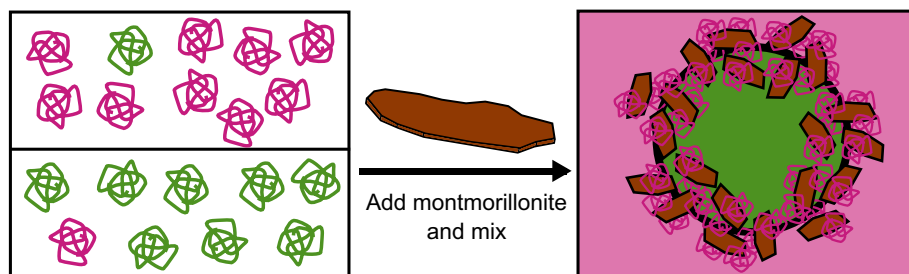
Stabilisation of water-in-water emulsions by montmorillonite platelets



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GRAPHICAL ABSTRACT



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ABSTRACT

The formation of water-in-water emulsions from the aqueous two phase system containing polyethylene oxide and pullulan, stabilised by montmorillonite platelets, was investigated. A novel approach of preparing the emulsions at non-equilibrium polymer concentrations was successfully utilised to control viscosity during mixing and allow the use of low energy emulsification methods. Polyethylene oxide adsorbed to the platelets much more strongly than pullulan favouring the formation of pullulan-in-polyethylene oxide emulsions which remained stable for a period of weeks. Polarising microscopy and small angle light scattering were used to show that droplets were most likely stabilised against coalescence by the adsorption of randomly oriented aggregates of platelets and against creaming by the formation of chains of droplets bridged by the adsorbed aggregates. Montmorillonite platelets were therefore shown to stabilise water-in-water emulsions and their preference for emulsion type was driven by the adsorption of the polymers to the particle surface.

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1. Introduction

Traditional emulsions are formed from the dispersion of one fluid into another highly chemically dissimilar one, such as hydrocarbon oils or lipids into water. The emulsion droplets are stabilised by the addition of species that can lower the surface energy such as surfactants, polymers and solid particles. In some

applications, such as in the production of low calorie foods or the encapsulation of sensitive active ingredients, the use of an oil phase is problematic and replacement with another aqueous phase would be more desirable. This can be achieved by using a system of water soluble polymers which separate into two aqueous phases above a certain concentration due to a positive mixing enthalpy and small mixing entropy [1]. These are known as aqueous two phase systems (ATPS) and have traditionally found use in the separation of biomolecules where a target entity with a greater affinity for one of the aqueous phases can be extracted without the use of hydrophobic phases to which it may be sensitive [2].

The majority component in these systems is water and the interfacial tension is typically many times lower than that of an

Abbreviations: ATPS, aqueous two phase system; PEO, polyethylene oxide; Pu, pullulan; SALS, small angle light scattering.

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oil/water interface (of order $\mu\text{N m}^{-1}$ as opposed to mN m^{-1}) [1]. The total polymer concentration across a water/water interface at equilibrium falls to a minimum in the centre of the interface and the concentration gradient typically extends over a few tens of nanometres [3]. The water-enriched interface is permeable to small solutes (such as ions and surfactants) meaning that to arrest the phase separation of the polymer phases and form an emulsion larger stabilisers, such as colloidal particles, are needed. This approach has been applied successfully and many different types of particle have now been reported to accumulate at the interface between two phase separated aqueous polymer solutions including fats [4], latex particles [5], proteins [6,7], liposomes [8], mineral platelets [9], cellulose nanocrystals [10] and bacteria [11].

One of the first systematic studies into the stabilisation of water-in-water emulsions by solid particles showed that accumulation of particles at the interface does not always result in a stable emulsion [12]. The low adsorption energy means that particles can be thrown off mechanically [12,6,13] and therefore interactions between particles at the interface and the formation of a robust interfacial layer is perhaps more important in the stabilisation of water-in-water emulsions than it is for oil-in-water emulsions [14,5,7].

The increase in number of studies into the stabilisation of water-in-water emulsions by particles has warranted the publication of two recent review papers [15,16] and there are some additional phenomena that are worth noting. The first is the possibility of the polymers comprising the two separated phases adsorbing onto the surface of the stabilising particle and altering its wettability *in situ*. This has been reported to result in an asymmetry in the type of emulsion that is favoured. For example dextran has been shown to adsorb more favourably to cellulose nanorods than polyethylene oxide resulting in particles which only stabilise polyethylene oxide-in-dextran emulsions [10]. Second is that the interaction with either phase can be highly dependent on environmental parameters. Protein stabilised water-in-water emulsions have been shown to be highly pH dependent and only form when the proteins complex with one of the polymers [6,17]. This response to external stimuli has been used to tune the behaviour of the emulsions where a change in pH can trigger destabilisation which may be useful in controlled delivery applications [13].

Non-spherical colloidal particles have the potential to be useful components in these all-aqueous formulations as they are well known to form complex structures in suspension [18] and have already been reported to stabilise water-in-water emulsions [9,10]. Detailed study of their organisation in these polymer-rich systems is therefore warranted as they have the potential to structure the continuous phase of the emulsions whilst also forming a robust stabilising layer at the water/water interface.

Here we take the smectite clay mineral montmorillonite, which can be exfoliated in aqueous suspension to form extremely high aspect ratio ($\sim 300:1$) platelets, and show that it can stabilise pullulan-in-polyethylene oxide emulsions. We use a combination of microscopy, rheology and light scattering to investigate the structures formed by the adsorbing particles and their stabilisation mechanisms. It was found that pullulan-in-polyethylene oxide emulsions were stable for at least 21 days, the adsorption of polyethylene oxide to the surface of the platelets drove an asymmetry in the type of emulsions that could be formed and the platelets were found to adsorb as clay-polymer aggregates.

2. Materials and methods

Wyoming montmorillonite (SWy-2) was purchased from the Clay Minerals Society source clays repository at Purdue

University. The composition of SWy-2 is $(\text{Si}_{7.94}\text{Al}_{0.06})(\text{Al}_{2.88}\text{Fe}_{0.5}\text{Mg}_{0.62})\text{O}_{20}(\text{OH})_4\text{Na}_{0.68}$ [19]. This montmorillonite has a cation exchange capacity of 84 mequiv/100 g [20] and the platelets typically have diameters in the range 200–2000 nm and are 1 nm thick. Polyethylene oxide (PEO, $M_w = 10^5 \text{ g mol}^{-1}$) was purchased from Sigma Aldrich, cosmetic grade pullulan (Pu, $M_w = 9 \times 10^4 \text{ g mol}^{-1}$) was kindly donated by Hayashibara, acridine orange (55% dye content) was purchased from Acros Organics and reagent grade sodium chloride was purchased from Fisher.

Preparation of montmorillonite dispersions was based on a previously described method [21]. 45 g L^{-1} powdered clay was added to deionised water and stirred for 24 h. The suspension was then dialysed against aqueous NaCl (1 M) for 1 week changing the solution everyday, to remove unwanted ions such as Ca^{2+} , and then dialysed against deionised water, changing water every day, until the conductivity of the dialysate was below $5 \mu\text{S cm}^{-1}$. The dialysed suspension was then diluted to 1 wt.% and left for 24 h for large impurities such as sand and quartz to settle out. The supernatant was concentrated up by evaporation and used as stock. Our previous work has shown that this results in complete exfoliation of the particles [22].

Polymer solutions were prepared by adding the solid polymer to deionised water and gently rolling at room temperature for a minimum of 12 h. PEO solutions were additionally centrifuged at 20,100g for 60 min in a Labnet Prism C2500 micro-centrifuge to remove silica impurities following a previously reported method [12]. The removed sediment accounted for no more than 2% of the total polymer mass.

Pu/PEO solutions for phase diagram determination were prepared by dissolving Pu into purified PEO solutions at different mass ratios by gentle rolling at room temperature for a minimum of 12 h. Once dissolved the solutions were left to stand in an incubator at 25 °C for 24 h and the presence or absence of a macroscopically visible fluid-fluid interface was noted.

Adsorption of the polymers onto montmorillonite was determined by adding stock montmorillonite suspension to stirring Pu and PEO solutions. The final montmorillonite concentration was 0.2 wt.% and the mass ratio of polymer to clay varied between 1 and 3. The montmorillonite-polymer mixtures were then rolled gently for 24 h and the unadsorbed polymer removed by centrifugation at 20,100g for 30 min in a Labnet Prism C2500 micro-centrifuge. The sedimented particles were then washed with deionised water, air dried and the adsorbed amount calculated by measuring the increase in carbon content using a Euro EA3000 elemental analyser.

Water-in-water emulsions were prepared by combining PEO and Pu solutions (prepared as detailed above) at 27 wt.% and 11 wt.% respectively. The Pu solution was first mixed with stock montmorillonite suspension and NaCl solution (final NaCl concentration in the emulsions was fixed at 0.01 M for all samples tested) by vortex mixing at 3000 rpm for 30 s and then gently rolled at room temperature for 60 min. The solutions were then combined and emulsified by vortex mixing at 3000 rpm for 60 s. The emulsion type was verified as Pu-in-PEO using the drop test where a droplet of emulsion (after being left to equilibrate) was pipetted onto a droplet of either 11 wt.% PEO or 20 wt.% Pu. An example is shown in Fig. 1 where the emulsion can be seen to have mixed with the PEO solution but not the Pu solution showing that the PEO-rich phase is the continuous phase.

The equilibrium Pu-rich phases are much more viscous than the PEO-rich phase making it difficult to shear the droplets sufficiently to achieve break-up using low energy methods such as hand shaking or vortex mixing. To achieve more reproducible results the phases were made at initially non-equilibrium compositions where the Pu-rich phase was less viscous, emulsified and then left to

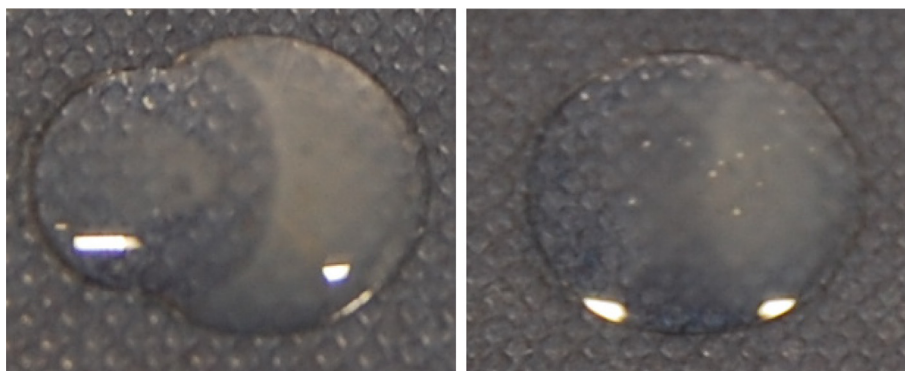


Fig. 1. Photographs of drop test of 6/7.6 wt.% Pu/PEO emulsion with 20 wt.% Pu solution (left) and 11 wt.% PEO solution (right).

equilibrate. The time-scale of equilibration and emulsion stability are discussed below.

A drop of each emulsion was then sealed between a glass microscope slide and cover slip using epoxy resin and imaged using an Olympus BX51 optical microscope and Pixelink PL-B62CU colour CCD camera using bright field (BF) and polarising (POM) optics over a period of 21 days. Emulsions were sealed in glass vials and photographed over a similar period of time. All samples were left for 24 h to equilibrate before commencing observations. Droplet size histograms were produced by counting a minimum of 100 droplets from each sample using Linear Intercept software (TU Darmstadt).

An analogous emulsion where the platelets had been dyed with acridine orange (0.0275 g dye per 1 g montmorillonite) was imaged using a Leica DMI6000 inverted epifluorescence microscope with a Leica DFC365FX monochrome CCD camera. The dye was found to have no impact on emulsion stability despite the presence of ~ 1 mM ZnCl_2 from the dye.

Small angle light scattering (SALS) was measured using home-built apparatus based on the designs of Verhaegh et al. [23] and Schätzel and Ackerson [24] using a 5 mW, $\lambda = 632.8$ nm He-Ne laser. Details of the instrument can be found in a previous publication [21]. Wavevectors from 10^{-4} nm^{-1} to $2 \times 10^{-3} \text{ nm}^{-1}$ were probed.

Rheological measurements were carried out using a Malvern Kinexus Pro rheometer with 20 mm parallel plates at a 1 mm gap and temperature of 20 ± 0.1 °C. Samples were preconditioned by resting for 10 min after loading. Equilibrium flow curves were measured in controlled rate mode from 0.1 to 500 s^{-1} . Oscillatory frequency sweeps were carried out in controlled strain mode with a maximum strain (γ_0) of 1% at frequencies from 1 to 10 Hz.

3. Results

3.1. Characterisation of the ATPS

The phase diagram of the Pu/PEO system was measured by preparing three Pu/PEO solutions at concentrations of 2/8.3 wt.%, 10/6 wt.% and 8.7/1.7 wt.% and sequentially diluting them with deionised water until there was no macroscopically visible interface present after 24 h. The results are shown in Fig. 2. The critical point for this mixture can be estimated as being around 3 wt.% Pu and 1 wt.% PEO. Emulsions for the main part of the experiment were prepared far from the critical point along a single tie-line indicated by the triangles and dotted line in Fig. 2. The tie-line was measured by iteratively preparing mixtures, allowing them to separate and calculating the weight fraction in the separated layers using partial specific volumes of 0.63 and $0.83 \text{ cm}^3 \text{ g}^{-1}$ for

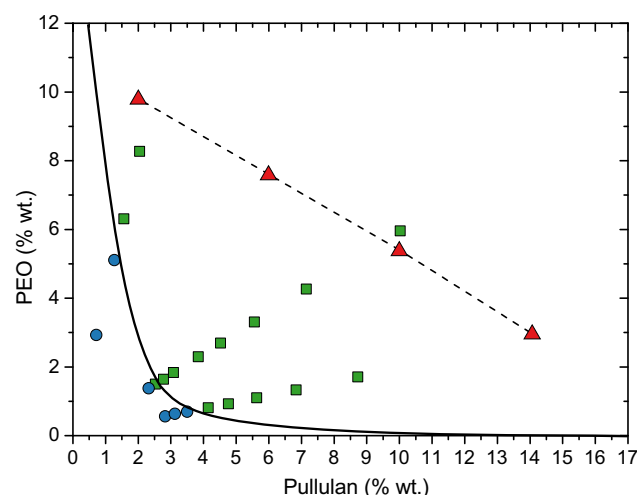


Fig. 2. Phase diagram of Pu-PEO mixtures. Squares represent phase separated compositions, circles represent homogeneous compositions and solid line is a guide to the eye. Dotted line represents a tie-line and triangles are compositions investigated for emulsion preparation.

Pu and PEO respectively [25,26,13]. The tie-line was selected to result in a sufficiently high interfacial tension to favour adsorption of the clay particles.

The interfacial tension along the tie-line was measured using the droplet relaxation method to be $98 \pm 13 \mu\text{N m}^{-1}$ (see [supplementary information](#) for details of measurement). Previous reports have shown the stabilisation of water-in-water emulsions with similar interfacial tensions [12,8,27,10,9].

After phase separation the Pu-rich phase was much more viscous than the PEO-rich phase and therefore difficult to emulsify by vortex mixing. To overcome this, the emulsions were prepared from polymer solutions at non-equilibrium concentrations where the viscosity of the Pu-rich phase was low enough to easily be emulsified by vortex mixing. The time scale of equilibration was monitored by adding a droplet of dilute Pu solution (7 wt.%) to a concentrated PEO solution (27 wt.%) using a microsyringe and monitoring the size of the droplet over time, once it had relaxed to a spherical shape, by microscopy. Fig. 3 shows the relative fall in the Pu droplet radius as water diffused into the continuous phase and the composition of the phases fell onto a tie-line parallel to that shown in Fig. 2. The time scale associated with this equilibration was of order 1000 s. Emulsification would therefore occur over the mixing period of 30 s and be followed by a period of around 1000 s where water egressed from the Pu droplets resulting in a slight initial reduction in droplet diameter. It should be noted

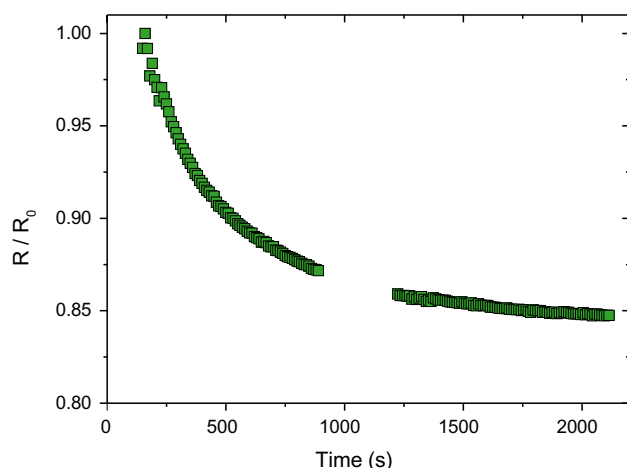


Fig. 3. Relative change in radius of a Pu droplet formed with in a PEO phase. Initial Pu and PEO concentrations were 7 wt.% and 27 wt.% respectively.

that in a more crowded system coalescence would also affect the droplet size during the period after emulsification.

3.2. Adsorption of polymers to the particle surface

Previous studies have shown that the phase separating polymers in water-in-water emulsions can adsorb to the stabilising particles to different extents resulting in an asymmetry in the affinity of the particles for the liquid phases [12,6,5]. The adsorption of Pu and PEO to montmorillonite was measured by monitoring the increase in carbon content of clay-polymer mixtures after removing unadsorbed polymer by centrifugation. Table 1 shows that Pu adsorbs to a very small extent and PEO adsorbs strongly with a saturation concentration of around 500 mg g^{-1} . A previous study of the adsorption of polyethylene oxide onto montmorillonite [28] found surface saturation concentrations of around 200 mg g^{-1} . That study used smaller molecular weight polymers but found a trend of increasing adsorption with molecular weight due to an increased tendency for the polymer to form loops at the surface. This may be the cause of the higher adsorbed amount shown in Table 1. A study of much larger molecular weight PEO (of order 10^5 g mol^{-1}) found dramatic shear thickening behaviour at low adsorbed amounts and stable suspensions that did not shear thicken at high adsorbed amounts similar to those studied here [29]. The polymers in the emulsions are present in excess therefore it is expected that the particles will be coated with a surface layer composed primarily of PEO.

The structure of a montmorillonite suspension in water at 0.01 M NaCl and a montmorillonite suspension in 7.6 wt.% PEO at 0.01 M NaCl were compared using SALS and this is shown in Fig. 4. At high Q the suspensions with and without polymer show slightly different scaling of scattered intensity with wavevector. Previous studies utilised SALS to show that montmorillonite platelets in aqueous suspension form fractal clusters [30,31]. This difference in high Q signal suggests a difference in the local arrangement

of the particles within the aggregates. At lower Q the suspension with polymer shows an upturn at around $2 \times 10^{-4} \text{ nm}^{-1}$ suggesting large extended structures with a minimum length scale of around $30 \mu\text{m}$. Detailed structural information of this larger scale structure cannot be verified without probing to lower Q . A Guinier analysis is shown in the supplementary information giving primary radii of gyration of around 1700 nm but evidence for further aggregation. The suspension without polymer does not show this low Q upturn meaning that in the absence of PEO the montmorillonite platelets assemble into much smaller structures.

3.3. Emulsion stability

Following the characterisation of the Pu/PEO system a series of emulsions were prepared along a single tie-line indicated by the triangles and dotted line in Fig. 2. Pu/PEO compositions of 2/9.8 wt.%, 6/7.6 wt.%, 10/5.4 wt.% and 14/3.2 wt.% were selected to result in Pu/PEO mass ratios after phase separation of approximately 10/90, 30/70, 50/50 and 70/30. All emulsions contained 1 wt.% montmorillonite and 0.01 M NaCl. Fig. 5 shows photographs of the 10/90, 30/70 and 50/50 systems 1 and 21 days after preparation. The 70/30 sample phase separated minutes after emulsification and photographs of this sample can be found in the supplementary information.

The 10/90 and 30/70 emulsions showed no macroscopic phase separation for at least 21 days. The 50/50 sample coarsened to a lower phase of macroscopically visible Pu droplets and an upper PEO phase containing the unadsorbed montmorillonite platelets forming a foam-like structure which did not fully phase separate over the course of the experiment. This suggests an asymmetry in the preference for emulsion type from the particles and is similar to the structure observed where zein particles were adsorbed at the water/water interface [32]. A previous study has shown that water-in-water emulsions can invert from one type to another as the dispersed phase volume fraction increases if the particles do not have a strong affinity for either phase [27]. Here this does not occur as PEO adsorbs onto the montmorillonite surface (as shown in Table 1) and increases the affinity of the particles for the PEO-rich phase favouring this as the continuous phase. In this case the formation of a PEO-in-Pu emulsion is completely disfavoured.

Fig. 6 shows bright field and polarising micrographs of the 30/70 emulsion after 21 days of standing in a vial. The droplet diameters are of order $10 \mu\text{m}$ and they appear to be forming linked

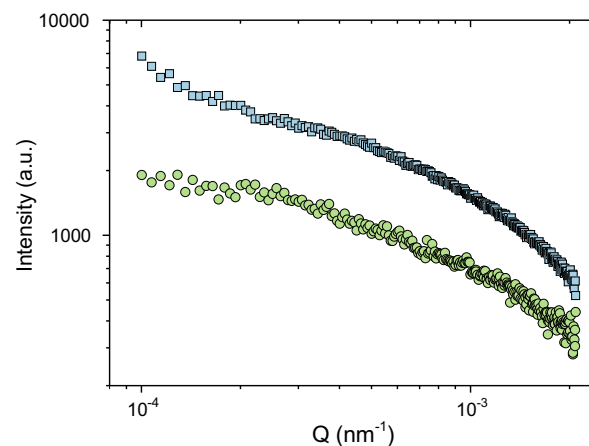


Fig. 4. SALS of 0.1 wt.% montmorillonite in water at 0.01 M NaCl (circles) and a montmorillonite suspension in 7.6 wt.% PEO at 0.01 M NaCl (squares). Intensity of upper dataset has been scaled by a factor of 2 for clarity.

Table 1
Adsorption of PEO and Pu to montmorillonite clay. $m_{\text{pol}}/m_{\text{mnt}}$ is the mass ratio of polymer to clay added and m_{ads} is the mass of polymer adsorbed per gram of clay.

Polymer	$m_{\text{pol}}/m_{\text{mnt}}$	m_{ads} (mg g^{-1})
Pu	1.1	93
PEO	1.25	424
PEO	2.8	521

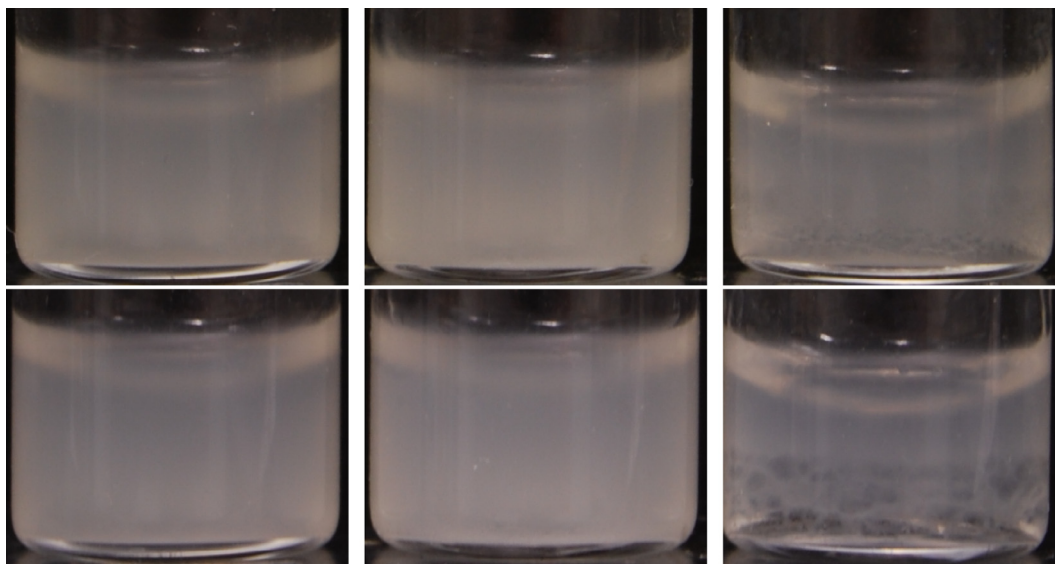


Fig. 5. Photographs of Pu-in-PEO emulsions at Pu/PEO ratios of 10/90 (left), 30/70 (middle) and 50/50 (right) 1 day after preparation (top) and 21 days after preparation (bottom).

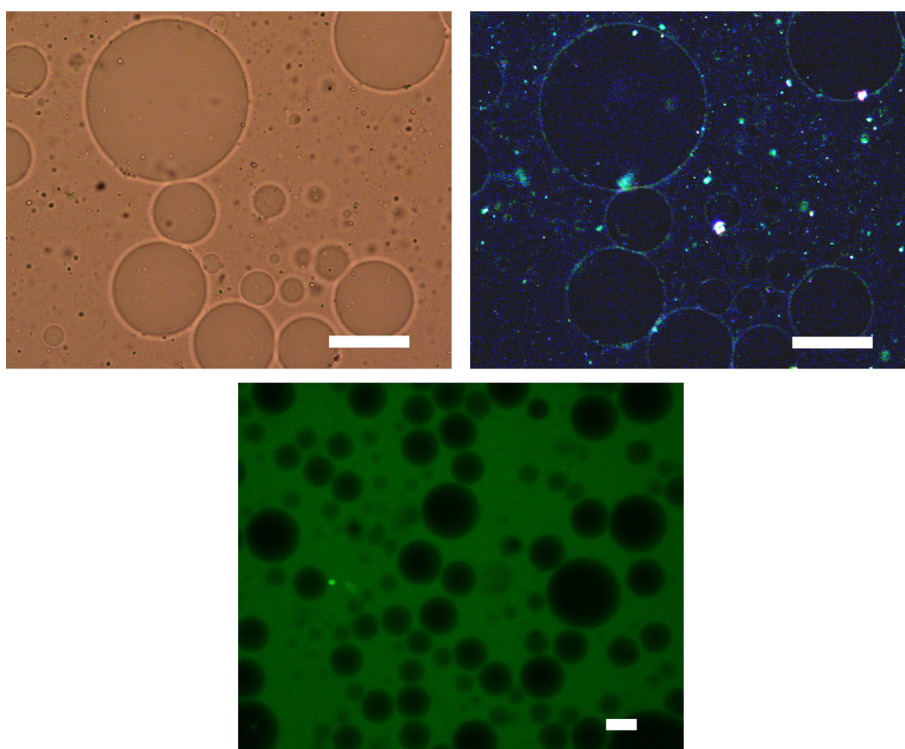


Fig. 6. Bright field (top left), polarising (top right) and fluorescence (bottom) microscopy images of 30/70 Pu-in-PEO emulsions. Contrast of polarising microscopy image has been enhanced for clarity. Scale bars represent 40 μm .

chains sharing slightly flattened edges. The polarising image shows birefringent objects in the continuous phase which may be montmorillonite aggregates (as evidenced in SALS in Fig. 4). See [supplementary information](#) for polarising optical microscopy of the emulsion and a clay suspension in the absence of polymer showing that the large amount of birefringent material observed in Fig. 6 is not present in the absence of polymer. There is also an accumulation of birefringent material at the droplet edges which we have previously observed for platelet stabilised oil-in-water emulsions [33,22]. It should be noted that in previous work on oil-in-water

emulsions the particle covered droplet edges showed strong birefringence and a cross pattern whereas here the birefringence is weak and approximately uniform around the edge suggesting a somewhat different configuration of adsorbed particles.

Fig. 6 also shows fluorescence microscopy of an emulsion of identical composition to emulsions imaged in the bright field and polarising micrographs only the montmorillonite has been dyed with acridine orange (2.75% of montmorillonite mass) which was found to have no effect on emulsion stability. The particles are completely excluded from the droplet phase which is possibly

enhanced by the strong adsorption of PEO onto the particles as shown in Table 1. Surprisingly, despite evidence for the accumulation of particles at the interface in polarising microscopy there is no visible accumulation fluorescent material in the fluorescence micrograph which has been shown in other studies to result in a bright ring around the droplets [13]. Our previous work on oil-in-water emulsions has shown that there is often a large excess of platelets remaining in the continuous phase after emulsification [22]. If this is the case here then the absence of a ring around the droplets in fluorescence microscopy may be due to a negligible difference between the interfacial and bulk platelet concentrations.

The coalescence stability of the 10/90 and 30/70 emulsions was followed over a period of 21 days. The surface weighted average droplet diameter (D_{32}) was measured and the results are shown in Fig. 7. There was little change in D_{32} of the 10/90 emulsion over the course of the experiment. The 30/70 sample showed an initial increase of 10 μm over the first week and then no subsequent change.

Fig. 8 shows an oscillatory frequency sweep of the emulsion continuous phase at a fixed maximum strain amplitude of 1% which is within the linear viscoelastic region. The viscous modulus is larger than the elastic modulus at all measured frequencies showing that the clay platelets do not form a gel in the continuous phase.

Fig. 9 shows equilibrium flow curves of the continuous phase and a 11 wt.% PEO solution at a shear rates from 0.1 to 500 s^{-1} . The platelets increase the viscosity and induce shear thinning behaviour when suspended in the Newtonian PEO solution. The low shear viscosity (η_m) of the montmorillonite suspension in PEO is taken as 2.2 mPa s which is the value at a shear rate of 0.1 s^{-1} .

The sedimentation velocity (v_{sed}) of an isolated 40 μm diameter droplet can be calculated by equating the buoyant and drag forces acting on a sphere suspended in a fluid medium [34]:

$$v_{\text{sed}} = \frac{2}{9} \frac{\Delta \rho r^2 g}{\eta_m} \quad (1)$$

where $\Delta \rho$ is the density difference between two phases, due to the high water content of the Pu-rich and PEO-rich phases this is expected to be around 0.05 g cm^{-3} [25,26,13], r is the droplet radius, g is the acceleration due to gravity and other symbols are as defined above. v_{sed} can therefore be estimated as $2 \times 10^{-8} \text{ m s}^{-1}$. An isolated droplet would take 2–3 days to sedi-

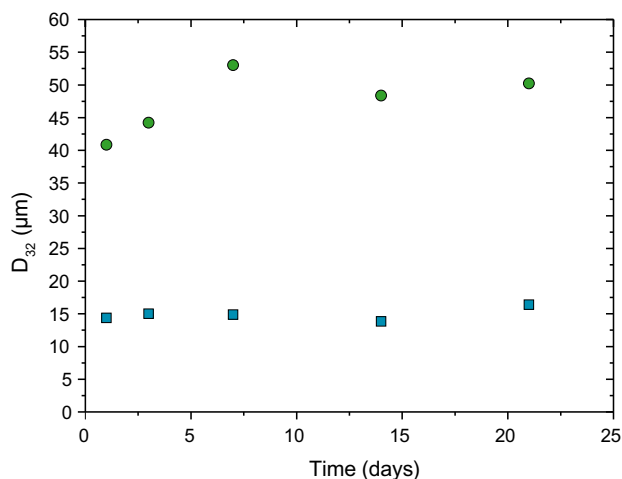


Fig. 7. Average droplet diameter (D_{32}) of Pu-in-PEO emulsions as a function of time. Pu/PEO ratios are 10/90 (squares) and 30/70 (circles).

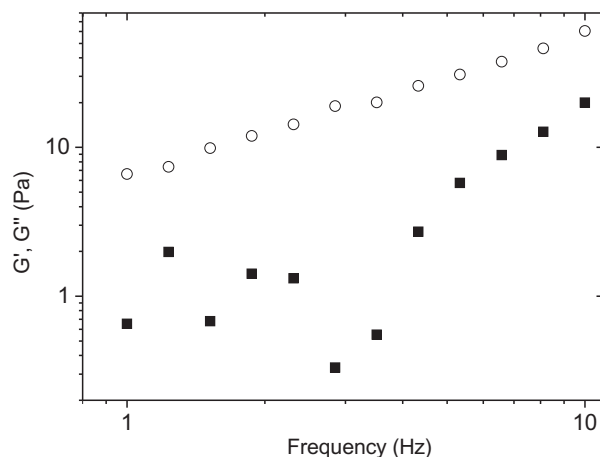


Fig. 8. Frequency sweep at fixed maximum strain of 1% of 1.4 wt.% montmorillonite in 11 wt.% PEO at 0.01 M NaCl. Viscous modulus indicated by open symbols and elastic modulus by closed symbols.

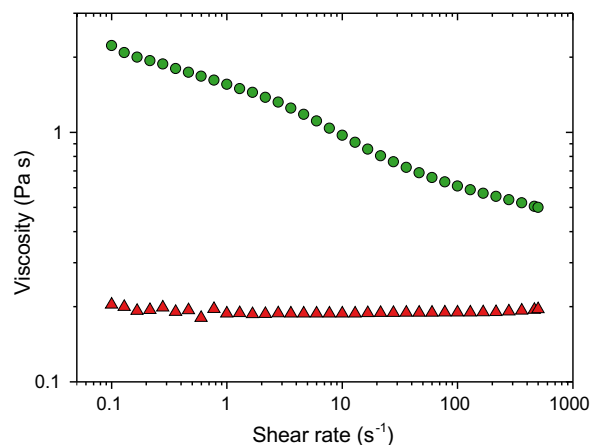


Fig. 9. Equilibrium flow curves of 11 wt.% PEO (triangles) and 1.4 wt.% montmorillonite in 11 wt.% PEO (circles). NaCl concentration was fixed at 0.01 M across all samples.

ment 0.5 cm which is the height of the liquid in the vials shown in Fig. 5. This is at odds with observations and is discussed below.

4. Discussion

The previous section showed that montmorillonite platelets can stabilise Pu-in-PEO emulsions and these remain stable to coalescence for at least 21 days. In this section the configuration of adsorbed particles and stabilisation mechanisms are discussed.

The presence of particles at the water/water interface replaces the energetically costly polymer concentration gradient. This favours the accumulation of particles at the interface and can stabilise the resulting layer against desorption due to thermal energy. Fig. 6 shows that there is an accumulation of birefringent material at the droplet edge in montmorillonite stabilised Pu-in-PEO emulsions. Previous similar observations have been used to show that montmorillonite platelets adsorb at the oil/water interface as a monolayer [33,22]. The birefringent pattern around the Pu-in-PEO droplets, however, is much weaker and does not show the fourfold pattern observed around the oil-in-water droplets. Fig. 10 shows the angular profiles around the edge of montmorillonite Pu-in-PEO and oil-in-water droplets observed between crossed polars.

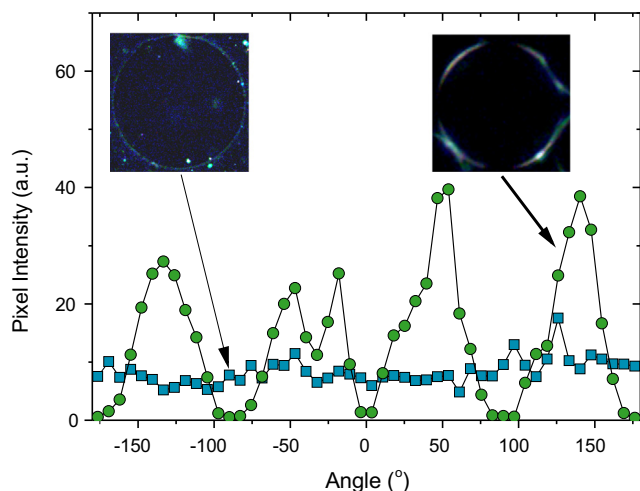


Fig. 10. Pixel intensity around the edge of a Pu-in-PEO droplet (squares) and a hexadecane-in-water droplet (circles) viewed between crossed polarisers. Images show the corresponding Pu-in-PEO droplet (left) and hexadecane-in-water droplet (right) [22]. Direction of polariser and analyser are vertical and horizontal. Contrast of images is enhanced for clarity.

The birefringence around the oil-in-water droplet oscillates and is at a maximum at 45° to the angle of the polariser or analyser and is zero at the angle of the polariser and analyser. As expected, this pattern remains upon rotation of the microscope slide. The birefringence around the Pu-in-PEO droplet is roughly equal around the entire circumference suggesting that at every point there is a population of platelets with some local ordering at approximately all orientations to produce the birefringence. The particles are therefore not arranged as a monolayer parallel to the droplet surface as has previously been reported for montmorillonite stabilised oil-in-water emulsions [33,22] and dextran-in-PEO emulsions stabilised by gibbsite platelets [9]. The lowest energy configuration for a platelet particle at a fluid interface has been calculated to be parallel to it [9], therefore there must be an additional interaction overcoming that of the interfacial energy for particles to not adsorb in this configuration.

We propose an aggregate structure which is composed of montmorillonite platelets coated with dense layers of PEO. The aggregation mechanism for this structure is unknown as the polymer concentration is too high for polymer chains to bridge particles together [34] and the depletion interaction is known to be weaker or even result in stabilisation at high polymer concentration [35]. Structures due to van der Waals interactions are unlikely as our previous work has utilised small angle X-ray scattering to show that electrostatic interactions at ionic strengths similar to that studied here are sufficient to prevent particle stacking which is known to result from these short range attractive interactions [22]. Additionally the polymer layer at the particle surface is thick enough to keep the particles beyond the range of van der Waals forces. The dense layer of large polymer chains adsorbed to the particle surface is also expected to stabilise the platelets against forming depletion induced stacked structures, which have been observed for montmorillonite platelets in the presence of a non-adsorbing polymer [36], favouring the formation of a more randomly ordered aggregate that retains some local order resulting in the uniform birefringence observed in Fig. 6. This structure falls in line with the small angle light scattering of dilute suspensions of montmorillonite in PEO solution observed in Fig. 4 which shows evidence for the formation of aggregates with length scales greater than $30\text{ }\mu\text{m}$. This is much greater than those observed in the absence of polymer where the maximum length scale is around $10\text{ }\mu\text{m}$ seen in Fig. 4 and our previous work [21]. The accumulation

of these randomly oriented platelet-polymer aggregates at the water/water interface would form a robust interfacial layer that stabilises the droplets against coalescence and result in the weak, uniform birefringence observed in Fig. 10. These structures are far from equilibrium and it is therefore possible that they may aggregate or gel further at the interface to form a robust stabilising layer.

It is however, not obvious why an aggregate composed of particles covered in PEO would adsorb to the interface with a phase that completely excludes PEO. Favourable adsorption would require the newly created solid/fluid interface to be of a lower energy than the fluid/fluid interface it is replacing. The presence of some montmorillonite at the surface of the aggregates may achieve this however, other mechanisms should be considered such as the particles being trapped by the high viscosity of the droplets. The identification of the mechanisms for interfacial accumulation would make interesting further work.

It has been reported that mechanical force can detach the particles from the water/water interface. This can be due to mechanical agitation of the entire sample or the action of coalescence [12,13,6]. Fig. 7 shows that the 30/70 Pu/PEO emulsion coalesced somewhat over the first week of storage but this only resulted in a $10\text{ }\mu\text{m}$ increase in diameter and no macroscopic phase separation. This may have been due to uncovered water/water interfaces of two droplets meeting and the mechanical action of relaxation to a spherical shape causing some of the particles to desorb.

The effects of coalescence events can be seen by observing the droplet shapes over long periods of time. After 14 and 21 days some of the droplets in the 30/70 emulsion adopted ellipsoidal shapes (see supplementary information). This is characteristic of an arrested coalescence event where jamming of particles at the interface acts to resist relaxation of the droplet back to a spherical shape. In this case the non-spherical shapes are not as extreme as those observed in some oil/water systems [37] suggesting that there is some competition between mechanical desorption and particle jamming at the interface. The 10/90 emulsion does not exhibit any notable change in droplet size (see Fig. 7) or non-spherical droplet shapes (see supplementary information) and this is most likely due to the larger separation between droplets at lower volume fraction and hence smaller probability of them coming into close enough proximity to coalesce.

It was shown in the previous section that the sedimentation velocity of a $40\text{ }\mu\text{m}$ diameter Pu droplet suspended in a continuous phase consisting of 1.4 wt.% montmorillonite dispersed in 11 wt.% PEO at 0.01 M NaCl should result in the droplets sedimenting to the bottom of the vial over a period of 2–3 days. Fig. 5 however shows no evidence for droplet sedimentation even after 21 days of storage at room temperature. Montmorillonite platelets in aqueous suspension have been reported to form gels [38] so could immobilise the droplets stabilising them to both sedimentation and coalescence. Upon measuring the viscous and elastic moduli of the continuous phase however, Fig. 8 shows that at the concentration present the platelets in the continuous phase do not form a gel so the stabilisation against sedimentation must occur by some other mechanism.

Fig. 6 shows evidence for the formation of chains of bridged droplets. This reinforcing effect of the droplets has already been reported in some particle stabilised water-in-water emulsions [9,10] and is well known for particle stabilised oil-in-water emulsions [39]. It occurs where the particles protruding into the continuous phase adsorb at the interface of two adjacent droplets and they appear to share a flat edge [40]. A chain of particles spanning the entire system could form a self-supporting network and stabilise the droplets against creaming or sedimentation. In the emulsions studied here Fig. 6 shows multiple droplets sharing slightly flattened edges suggesting that they are bridged by interfacial par-

ticles. This may therefore be the mechanism by which the emulsions are stabilised against sedimentation. It would be an interesting experiment to probe the rheological properties of these emulsion gels however, due to their sensitivity to mechanical agitation, this would require time resolved rheo-optical measurements to ensure the act of loading the sample onto the rheometer did not modify the structure. This warrants a dedicated rheological investigation which is beyond the scope of the study of emulsion formation and stabilisation presented here but would make interesting further work. It also follows that the particles must have formed a robust interfacial layer as bridging requires the droplets to be in close proximity which would result in coalescence if there were no strong interfacial barrier between the droplets.

Due to the low interfacial tension and therefore low adsorption energy of particles at the water/water interface it has been reported that the formation of an interfacial layer stabilised by attractive interactions between the adsorbed species is required to form an effective barrier to coalescence [5,14,16]. We have shown that in the presence of a high concentration of PEO montmorillonite platelets form aggregate structures that are much larger than those formed in the absence of polymer. They therefore show the correct characteristics to form a robust stabilising layer at the interface resulting in the formation of stable water-in-water emulsions observed in this work.

5. Conclusions

This study has investigated the stabilisation of water-in-water emulsions composed of a phase separating aqueous solution of pullulan and polyethylene oxide by montmorillonite platelet particles. Polyethylene oxide was found to adsorb strongly to the platelet surfaces and result in them being completely excluded from the pullulan phase. The platelets favoured the formation of Pu-in-PEO emulsions, as previously observed for particles which show a strong affinity for one of the polymers [10], and these were stable to both creaming and coalescence for at least 21 days. This adds montmorillonite to a growing number of water-in-water emulsion stabilisers [4,5,7–9].

The configuration of the particles was investigated by polarising microscopy and small angle light scattering which showed evidence for the adsorption of randomly oriented aggregates of polymer coated montmorillonite platelets. The stabilisation mechanisms were also investigated by microscopy and rheology of the continuous phase and it was found that droplet bridging was important in preventing sedimentation and the formation of a robust layer of particles at the interface stabilised the droplets against coalescence.

Now that the structure and stability of water-in-water emulsions stabilised by montmorillonite platelets has been established, further study of the impact of other parameters such as particle concentration, ionic strength and surface chemistry, which we have previously shown to drastically impact upon the structure of montmorillonite suspensions and oil-in-water emulsions [21,41], would make natural further work. For example it would be expected that ionic strength would control the length scale over which the particles interact electrostatically and could alter the aggregate structures and there may be a minimum threshold clay concentration at which the emulsions are stable to coalescence and also to creaming. Additionally, an alternative aqueous two phase system, where the particles had equal affinity to the two polymers, could be investigated as other similar studies have shown the inversion of one emulsion type to another in such systems [27]. It would also be interesting to investigate the structure of the particles adsorbed at the interface in other polymer systems

to identify whether the formation of particle aggregates is a common behaviour for montmorillonite platelets adsorbed at the water/water interface and use techniques such as small angle X-ray scattering to probe the intra-aggregate structure as it is unknown whether the birefringence of the aggregates is due to nematic-like structures or some other configuration of platelets.

High surface area platelet particles have shown the ability to stabilise water-in-water emulsions. In this case the interactions of the platelets with the polymers composing the aqueous phases are key in determining the behaviour. This adds to a growing tool kit of water-in-water emulsion stabilisers with the goal of eventually being able to target the properties for a particular application by the careful selection of a system of polymers and appropriate stabiliser.

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Appendix A. Supplementary material

Supplementary information includes measurement of the interfacial tension along the tie-line indicated in Fig. 2, micrographs of Pu-in-PEO emulsions from stability experiment shown in Fig. 7, corresponding droplet size histograms of these emulsions, polarising optical microscopy of a montmorillonite suspension for comparison with Fig. 6 and a Guinier analysis of the small angle light scattering data shown in Fig. 4. Raw rheology, small angle light scattering data and micrographs used to calculate droplet sizes in Fig. 3 are openly available from the University of Bristol Research Data Repository (DOI: <http://dx.doi.org/10.5523/bris.lhdk3q72ufj2fn19bu7phmgk>). Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcis.2017.05.062>.

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